



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

|  |           |   |
|--|-----------|---|
| (51) International Patent Classification <sup>5</sup> :<br>C07K 7/20, 7/26, 7/06<br>A61K 37/24, 37/43  | <b>A1</b> | (11) International Publication Number: <b>WO 91/18016</b><br>(43) International Publication Date: 28 November 1991 (28.11.91)   |
| (21) International Application Number: PCT/EP91/00727<br>(22) International Filing Date: 17 April 1991 (17.04.91)<br>(30) Priority data:<br>20273 A/90 11 May 1990 (11.05.90) IT<br>(71)(72) Applicant and Inventor: DEGHENGHI, Romano [IT/CH]; Chesaux Dessus B1, CH-1264 S.-Cergue (CH).<br>(74) Agent: MINOJA, Fabrizio; Studio Consulenza Brevettuale, Via Rossini, 8, I-20122 Milano (IT).<br>(81) Designated States: AT (European patent), AU, BB, BE (European patent), BF (OAPI patent), BG, BJ (OAPI patent), BR, CA, CF (OAPI patent), CG (OAPI patent), CH (European patent), CM (OAPI patent), DE (European patent), DK (European patent), ES (European patent), FI, FR (European patent), GA (OAPI patent), GB (European patent), GR (European patent), HU, IT (European patent), JP, KP, KR, LK, LU (European patent), MC, MG, ML (OAPI patent), MR (OAPI patent), MW, NL (European patent), NO, PL, RO, SD, SE (European patent), SN (OAPI patent), SU, TD (OAPI patent), TG (OAPI patent), US. |           | Published<br><i>With international search report.<br/>         Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i> |
| (54) Title: BIOLOGICALLY ACTIVE PEPTIDES CONTAINING D-2-ALKYLTRYPTOPHAN<br><br>(57) Abstract<br><p>Peptides containing at least one D-2-alkyltryptophan residue in their amino acid chain and having pharmacological activity similar to that of analogous peptides containing natural unsubstituted D-tryptophan residues in place of at least one D-2-alkyltryptophan. These new peptides are more resistant to oxidative degradation which usually takes place, for example, in the presence of reactive radicals or during high energy sterilization than unsubstituted tryptophan containing peptides.</p>  |           |   |

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

|    |                          |    |  |    |                          |
|----|--------------------------|----|--|----|--------------------------|
| AT | Austria                  | ES | Spain                                    | MG | Madagascar               |
| AU | Australia                | FI | Finland                                  | ML | Mali                     |
| BB | Barbados                 | FR | France                                   | MN | Mongolia                 |
| BE | Belgium                  | GA | Gabon                                    | MR | Mauritania               |
| BF | Burkina Faso             | GB | United Kingdom                           | MW | Malawi                   |
| BG | Bulgaria                 | GN | Guinea                                   | NL | Netherlands              |
| BJ | Benin                    | GR | Greece                                   | NO | Norway                   |
| BR | Brazil                   | HU | Hungary                                  | PL | Poland                   |
| CA | Canada                   | IT | Italy                                    | RO | Romania                  |
| CF | Central African Republic | JP | Japan                                    | SD | Sudan                    |
| CG | Congo                    | KP | Democratic People's Republic<br>of Korea | SE | Sweden                   |
| CH | Switzerland              | KR | Republic of Korea                        | SN | Senegal                  |
| CI | Côte d'Ivoire            | LI | Liechtenstein                            | SU | Soviet Union             |
| CM | Cameroon                 | LK | Sri Lanka                                | TD | Chad                     |
| CS | Czechoslovakia           | LU | Luxembourg                               | TC | Togo                     |
| DE | Germany                  | MC | Monaco                                   | US | United States of America |
| DK | Denmark                  |    |  |    |                          |

BIOLOGICALLY ACTIVE PEPTIDES  
CONTAINING D-2-ALKYLTRYPTOPHAN

Technical Field

The present invention relates to the field of biologically active peptides. Specifically, this invention relates to biologically active peptides containing the amino acid D-Tryptophan (D-Trp).

Background Art

It is well known that the incorporation or substitution of a D-Tryptophan residue into a biologically active peptide chain enhances the activity of that chain. Furthermore, such incorporation or substitution will prolong the biological activity. The prolonged and enhanced effectiveness of such peptides probably relates the increased resistance to degradation by peptidases.

Examples of D-Tryptophan containing peptides are the LHRH agonists as described by D.H. Coy et al., Journal of Medical Chemistry, volume 19, page 423 (1976), W. Koenig et al., Peptide Chemistry (1987), T. Shiba and S. Sakakibara (eds.), Osaka, Protein Research Foundation, Osaka (1988), page 591, B.J.A. Furr et al., Journal of Endocrinol. Invest., volume 11, page 535 (1988). Examples of D-Tryptophan containing somastostatin analogs, such as the peptides octreotide and vapreotide are disclosed by R. Deghenghi, Biomedicine and Pharmacotherapy, volume 42, page 585 (1988). Another example of a D-Tryptophan containing peptide are the synthetic antagonists of Substance P as disclosed by D. Regoli et al., European Journal of Pharmacology, volume 99, page 193, (1984), and GHRP-6 described by C.Y. Bowers et al., Endocrinology, volume 114, page 1537, (1984).

Peptides containing Tryptophan have been subject to degradation due to the "Kynurenine pathway". In this pathway, the enzyme Tryptophan pyrrolase (i.e., indolamine 2,3-dioxygenase) degrades the pyrrole ring of Tryptophan. Kynurenine and other breakdown products

- 2 -

are generated by this degradation. Some of the breakdown products have been shown to be toxic when present in elevated concentrations as reported by R.M. Silver et al., The New England Journal of Medicine,  
5 volume 322, page 874, (1990).

D-Tryptophan containing peptides are subject to degradation by oxygen and other reactive radicals as reported by R. Geiger and W. Koenig, "The Peptides," Academic Press, volume 3, page 82, New York (1981).  
10 The D-Tryptophan in the peptide chain may react with active or activated groups when peptides are formulated in certain controlled delivery pharmaceutical compositions, such as those based on  
15 polylactic-polyglycolic acid polymers. Such degradation is thought to be facilitated by either heat or by the presence of catalysts. It is also possible that radiolysis products formed during ionizing  
sterilization of these pharmaceutical compositions may facilitate the breakdown of D-Tryptophan. Clearly, the  
20 breakdown of D-Tryptophan, and the concomitant breakdown of the pharmaceutical compound containing D-Tryptophan is an undesirable effect.

What is needed is a derivative of D-Tryptophan which retains the prolonged and increased  
25 biological activity discussed above, while resisting degradation by indolamine dioxygenase, oxygen, or other reactive radicals. It is of course essential that such a derivative of D-Tryptophan would maintain biological activity as compared to natural D-Tryptophan containing  
30 bioactive peptides.

The terms "biological effect" or "pharmacological effect" as used in the present disclosure refer to the qualitative effect that a bioactive peptide has upon living tissue. As an  
35 example, LHRH, luteinizing hormone releasing hormone, has the biological effect of causing cells of the anterior pituitary gland to release luteinizing

- 3 -

hormone. In contrast, the term "potency" is used in its conventional sense to refer to the degree and duration of the bioactivity of a given peptide.

Utilizing these terms as defined above, what  
5 is needed is a Tryptophan containing bioactive peptide which is resistant to oxidative degradation and reactive radical attack while maintaining the same biological activity and a similar or greater potency than the presently available analogous peptides  
10 provide.

#### Summary of Invention

Now in accordance with the present invention, a derivative of D-Tryptophan has been discovered which  
15 imparts to a biologically active peptide incorporating the derivative improved resistance to oxidative breakdown reactions of the Tryptophan derivative, while maintaining the biological activity and pharmacological effect exhibited by peptides incorporating unaltered  
20 D-Tryptophan. Specifically, the present invention relates to biologically active peptides incorporating at least one D-Tryptophan in which a lower alkyl group, preferably an alkyl group containing 1 to 3 carbons, is substituted in the 2 position. Peptides incorporating  
25 such substituted D-Tryptophans are more stable in the presence of reactive radicals, or when pharmaceuticals containing such peptides are exposed to ionizing radiation.

#### 30 Detailed Description

Biologically active peptides in accordance with the present invention are characterized by the following formula:

35

A-D-2-ALK-TRP-B

---

- 4 -

- where alk is a lower alkyl group, preferably comprising 1 to 3 carbons. A is SEQUENCE ID NO:1, wherein the Glutamic Acid residue at SEQUENCE position 1 is Pyro-Glu; D-Phe-Cys-Phe; SEQUENCE ID NO:2 wherein the
- 5 Phenylalanine residue at SEQUENCE position 1 is D-Phe, the Proline residue at SEQUENCE position 4 is D-Proline and the Tryptophan residues at SEQUENCE positions 7 and 9 are D-Tryptophan; Arg-D-Trp-N-methyl-Phe; or His; and B is SEQUENCE ID NO:3 wherein the Glycine residue
- 10 at sequence position 4 is Gly-NH<sub>2</sub>; Leu-Arg-Pro-NHCH<sub>2</sub>CH<sub>3</sub>; Lys-Thr-Cys-NHCH(CH<sub>2</sub>OH)CHOH(CH<sub>3</sub>); SEQUENCE ID NO:4 wherein the Tryptophan residue at SEQUENCE position 4 is Trp-NH<sub>2</sub>; Met-NH<sub>2</sub>; Leu-Met-NH<sub>2</sub>; SEQUENCE ID NO:5 wherein the Phenylalanine residue at SEQUENCE position
- 15 3 is D-Phe and the Lysine residue at SEQUENCE position 4 is Lys-NH<sub>2</sub>; provided that when A is SEQUENCE ID NO:1, B is SEQUENCE ID NO:3 wherein the Glycine residue at SEQUENCE position 4 is Gly-NH<sub>2</sub> or Leu-Arg-Pro-NHCH<sub>2</sub>CH<sub>3</sub>; when A is D-Phe-Cys-Phe, B is
- 20 Lys-Thr-Cys-NHCH-(CH<sub>2</sub>OH)CHOH(CH<sub>3</sub>) wherein the cysteine units are bound to each other by a cyclic disulphide; when A is D-Phe-Cys-Tyr, B is SEQUENCE ID NO:4 wherein the cysteine units are bound to each other by a cyclic disulfide and the Tryptophan residue at SEQUENCE
- 25 position 4 is Trp-NH<sub>2</sub>; when A is SEQUENCE ID NO:6 wherein the Proline residue in SEQUENCE position 1 is D-Pro and the Tryptophan residues at SEQUENCE positions 4 and 6 are D-Trp, B is Met-NH<sub>2</sub>; when A is Arg-D-Trp-n-methyl-Phe, B is Leu-Met-NH<sub>2</sub>; or when A is
- 30 His, B is SEQUENCE ID NO:5 wherein the Phenylalanine residue at SEQUENCE position 3 is D-Phe and the Lysine residue at SEQUENCE position 4 is Lys-NH<sub>2</sub>.

The following compounds are biologically active peptides of the present invention which contain

35 at least one C-2 substituted D-Tryptophan according to the present invention.

- 5 -

A. SEQUENCE ID NO:7 wherein the Glutamic acid residue at SEQUENCE position 1 is Pyro-Glu, the Tryptophan residue at SEQUENCE position 6 is D-2-methyl-Trp, and the Glycine residue at SEQUENCE  
5 position 10 is Pro-Gly-NH<sub>2</sub>.

B. SEQUENCE ID NO:8 wherein the Glutamic Acid residue at SEQUENCE position 1 is Pyro-Glu, the Tryptophan residue at SEQUENCE position 6 is D-2-methyl-Trp, and the Proline residue at SEQUENCE  
10 position 9 is Pro-NHCH<sub>2</sub>CH<sub>3</sub>.

C. SEQUENCE ID NO:9 wherein the Proline residue at SEQUENCE position 1 is D-Pro, the Tryptophan residue at SEQUENCE position 4 is D-Trp, the Tryptophan residue at SEQUENCE position 6 is D-Trp, the Tryptophan  
15 residue at SEQUENCE position 7 is D-2-ethyl-Trp, and the Methionine residue at SEQUENCE position 8 is Met-NH<sub>2</sub>.

D. SEQUENCE ID NO:10 wherein the Tryptophan residue at SEQUENCE position 2 is D-Trp, the  
20 Phenylalanine residue at SEQUENCE position 3 is N-methyl-Phe, the Tryptophan residue at SEQUENCE position 4 is D-2-isopropyl-Trp, and the Methionine residue at SEQUENCE position 6 is Met-NH<sub>2</sub>.

E. SEQUENCE NO:11 (and its cyclic oxidation  
25 product) wherein the Phenylalanine residue at SEQUENCE position 1 is D-Phe, the Tryptophan residue at SEQUENCE position 4 is D-2-methyl-Trp and the Cysteine residue at SEQUENCE position 7 is Cys-NHCH(CH<sub>2</sub>OH)CHOHCH<sub>3</sub>.

F. SEQUENCE ID NO:12 (and its cyclic  
30 oxidation product) wherein the Phenylalanine residue at SEQUENCE position 1 is D-Phe, the Tryptophan residue at SEQUENCE position 4 is D-2-methyl-Trp, and the Tryptophan residue at SEQUENCE position 8 is Trp-NH<sub>2</sub>.

G. SEQUENCE ID NO:13 wherein the Tryptophan  
35 residue at SEQUENCE position 2 is D-2-methyl Trp, the

- 6 -

Phenylalanine residue at SEQUENCE position 5 is D-Phe, and the Lysine residue at position 6 is Lys-NH<sub>2</sub>.

Compounds A and B are analogs of the natural peptide SEQUENCE ID NO:14 wherein the Glutamic acid residue at SEQUENCE position 1 is Pyro-Glu and the Glycine residue at position 10 is Gly-NH<sub>2</sub>. SEQUENCE ID NO:14 is luteinizing hormone releasing hormone (LH-RH), a neurohumoral hormone produced in the hypothalamus which stimulates the secretion of the LH luteinizing hormone by the anterior pituitary gland. Compounds A and B pertain therefore to the class of LHRH agonists and are also defined respectively as follows: [D-2-methyl-Trp<sup>6</sup>]LHRH and [Des-Gly<sup>10</sup>-D-2-methyl-Trp<sup>6</sup>-Pro-ethylamide<sup>9</sup>]LHRH.

Compounds C and D are antagonists of substance P. Substance P is a neurotransmitter used by sensory neurons that convey responses of pain or other noxious stimuli to the central nervous system. Compounds C and D therefore have analgesic and anti-inflammatory effects. Peptides E and F are analogs (agonists) of somatostatin and as such show antisecretory and antitumoral activity. Peptide G is an analog of GHRP (Growth Hormone Releasing Peptide) stimulating the release of growth hormone.

2-methyl-Tryptophan is known (cf. H.N. Rydon, J. Chem. Soc. 1948, 705) and the homologous alkylated derivatives are conveniently prepared from the corresponding 2-alkyl indoles by well known methods (cf. J.P. Li et al., Synthesis (1), 73, 1988). The resolution of the racemic Tryptophan derivatives to give the D-enantiomers of the present invention can also be achieved by a variety of methods (cf. Amino Acids, Peptides and Proteins, Vol. 16, pages 18-20, The Royal Society of Chemistry, London, 1985). Both the solution phase or the solid phase method of peptide synthesis can be used to make the peptides of this invention, (cf. R. Geiger et al., "The Peptides",



Academic Press, New York 1981). If the solid phase method is used, peptide synthesizers such as the Applied Biosystem 430A, Bioresearch Sam 9500 or the Beckman Model 990 are preferably used.

- 5           According to this methodology, the first amino acid is linked to the benzhydrylamine resin and the remaining protected amino acids are then coupled in a step wise manner using the standard procedures recommended by the manufacturers of the synthesizers.
- 10          For instance, amino acid couplings are performed by using symmetrical anhydrides in the Applied Biosystems Synthesizer and diisopropylcarbodiimide in the Bioresearch or Beckman machines. The amino acid derivatives are protected by the tertiary
- 15          butoxy-carbonyl groups on the alpha-amino function during the synthesis. The functional groups present in the amino-acid in the side chain are previously protected, e.g. by acetyl (Ac), benzoyl (Bz), t-butyloxycarbonyl (Boc), benzyloxymethyl (Bom), benzyl
- 20          (Bzl), benzyloxycarbonyl (Z), formyl (For), p-nitro-phenyl ester (ONp), tosyl (Tos), etc. For instance, the functional groups of Histidine are protected by benzyloxymethyl (His(Bom)), tosyl (His(Tos)), the functional groups of Tryptophan by
- 25          formyl (Trp(For)), those of Serine by benzyl (Ser(Bzl)), those of Tyrosine by 2-Br-benzyloxycarbonyl (Tyr(2-Br-Z)), those of Arginine by tosyl (Arg(Tos)), those of Leucine by O-benzyl-p-tosyl (Leu(O-Bzl-p-Tos)), those of Proline by O-benzyl HCl (Pro(O-Bzl HCl)), those of Glycine by O-benzyl HCl (Gly (O-Bzl
- 30          HCl)), those of Cysteine by 4-methyl-benzyl (Cys(4-Me-Bzl)), those of Lysine by benzyloxycarbonyl (Lys(Z)), those of Threonine by benzyl-OH (Thr(Bzl-OH)), those of Valine by O-benzyl-tosyl
- 35          (Val(O-Bzl-p-Tos)), those of Glutamic Acid by O-benzyl (Glu(O-Bzl)), those of Methionine by P-nitrophenyl

ester (Me(Onp)), and those of Alanine by O-benzyl HCl (Ala(O-Bzl HCl)).

The Boc protective groups on the alpha-aminic function are removed at each stage by treatment with  
5 60% trifluoroacetic acid ("TFA") in dichloromethane. Cleavage of Trp and Met containing peptides from the resin with simultaneous removal of all side-chain protecting groups is achieved as described by J.P. Tam et al., J. Am. Chem. Soc., Vol 105, page 6442 (1983).  
10 The crude peptides after HF cleavage are purified on a Sephadex G-50 F column in 50% acetic acid or by preparative reverse phase HPLC using gradients of acetonitrile and water containing 0.1% trifluoroacetic acid.

15 The examples that follow are given for illustrative purposes only, but are not limitative of the present invention.

#### Example 1

20 SEQUENCE ID NO:7 wherein the Glutamic acid residue at SEQUENCE position 1 is Pyro-Glu, the Tryptophan residue at SEQUENCE position 6 is D-2-methyl-Trp, and the Glycine residue at SEQUENCE position 10 is Gly-NH<sub>2</sub>.

25 The protective groups for the side chains are Tosyl (Tos) for Arginine and Histidine and Bromo-benzylloxycarbonyl (2-Br-Z) for Tyrosine. The benzhydrylamine resin (2.2g) (Bachem<sup>®</sup>), was cross-linked at 1% with Prolinc and the apparatus used was a Beckman  
30 Model 990. The amino acids protected by Boc (tert-butyloxycarbonyl) are coupled with dicyclohexylcarbodiimide. The Boc groups are removed by trifluoroacetic acid in methylene chloride.

The synthesis yielded 4.07 g of the  
35 decapeptide-resin (98% of theoretical weight gain). Part of this resin (1.5 g) was stirred at 0° centigrade for 30 minutes with HF (24 ml) and anisole (8 ml). HF

- 9 -

was then removed as rapidly as possible (ca. 60 min) in vacuo and EtOAc was added to the thus obtained residue. Solid material was filtered, washed with EtOAc, dried, and extracted with 2 M AcOH. Lyophilization gave a  
5 white powder which was purified by gel filtration on a column (2.5 X 95 cm) of Sephadex G-25 (fine) by elution with 2 M AcOH. The eluate portion corresponding to the major peak was then dried and eluted further on a column (2.5 X 95 cm) of Sephadex G-25 (fine) previously  
10 equilibrated with the lower phase followed by the upper phase of the following biphasic solvent mixture n-BuOH-AcOH-H<sub>2</sub>O (4:1:5). Elution with the upper phase gave a major peak and the peptide from this area was collected, concentrated to dryness, and lyophilized  
15 from dilute AcOH to give the titled peptide as a white powder. Amino acid analysis was consistent with the desired structure.

#### Example 2

20 SEQUENCE ID NO:8 wherein the Glutamic Acid residue at SEQUENCE position 1 is Pyro-Glu, the Tryptophan residue at SEQUENCE position 6 is D-2-methyl-Trp, and the Proline residue at SEQUENCE position 9 is Pro-NHCH<sub>2</sub>CH<sub>3</sub>.

25 The peptide was assembled on a 1% cross-linked Pro-Merrifield resin (2.0 g, 1.0 mmol of Pro) using the same conditions and protecting groups employed in Example 1, with the exception that dinitrophenol group protection was used for the  
30 imidazole group of histidine. The peptide-resin obtained (3.45 g) was stirred with ethylamine (20 ml, 0°C) for 6 hours and excess amine was removed in vacuo. The protected peptide resin was extracted with MeOH and precipitated by the addition of a large excess of EtOAc  
35 to give 1.36 g of material. The obtained product was treated and deprotected with HF-anisole and crude peptide obtained after this treatment was purified by

---

gel filtration followed by partition chromatography to yield the homogeneous peptide cited. Amino acid analysis was consistent with the desired structure.

5 Examples 3-7

Using the above described methods with appropriate modifications well known to the skilled in the art, the following peptides are synthesized:

SEQUENCE ID NO:9 wherein the Proline residue  
10 at SEQUENCE position 1 is D-Proline, the Tryptophan residues at SEQUENCE positions 4 and 6 are D-Trp, the Tryptophan residue at SEQUENCE position 7 is D-2-ethyl-Trp, and the Methionine residue at SEQUENCE position 8 is Met-NH<sub>2</sub>.

15 SEQUENCE ID NO:10 wherein the Tryptophan residue at SEQUENCE position 2 is D-Trp, the Phenylalanine residue at SEQUENCE position 3 is N-methyl-Phe, the Tryptophan residue at SEQUENCE position 4 is D-2-isopropyl-Trp, and the Methionine residue at  
20 SEQUENCE position 6 is Met-NH<sub>2</sub>.

SEQUENCE ID NO:11 wherein the Phenylalanine residue at SEQUENCE position 1 is D-Phe, the Tryptophan residue at SEQUENCE position 4 is D-2-methyl-Trp, and the Cysteine residue at SEQUENCE position 7 is Cys-  
25 NHCH(CH<sub>2</sub>OH)CHOHCH<sub>2</sub> (cyclic disulphide).

SEQUENCE ID NO:12 wherein the Phenylalanine residue at SEQUENCE position 1 is D-Phe, the Tryptophan residue at SEQUENCE position 4 is D-2-methyl-Trp, and the Tryptophan residue at SEQUENCE position 8 is Trp-NH<sub>2</sub>,  
30 (cyclic disulphide).

SEQUENCE ID NO:13 wherein the Tryptophan residue at SEQUENCE position 2 is D-2-methyl-Trp, the Phenylalanine residue at SEQUENCE position 5 is D-Phe, and the Lysine residue at SEQUENCE position 6 is Lys-  
35 NH<sub>2</sub>.

- 11 -

Although the aforementioned examples of the present invention disclose specific embodiments thereof, it is believed that the substitution of an D-2-alkylTryptophan in bioactive peptide containing at least one Tryptophan residue will yield bioactive peptides providing the advantages and benefits discussed above.

The incorporation of a D-2-alkylTryptophan in bioactive peptides as described above provides a method for prolonging and preserving the activity of such peptides. When analogous bioactive peptides not substituted with an D-2-alkylTryptophan are exposed to various processing conditions and substances, the activity of such peptides may be adversely effected. Sterilizing procedures used in the pharmaceutical industry may expose bioactive compounds to ionizing radiation. Such radiation may effect the formation of reactive radicals. Tryptophan containing peptides are particularly susceptible to attack by such radicals and such attack may render the peptide ineffective, or possibly toxic. Furthermore, various formulating compounds, such as polylactic-polyglycolic acid polymers may contain active, or activated groups which may also attack Tryptophan containing bioactive peptides. The present invention provides a method for protecting a tryptophan containing bioactive peptide from these manufacturing hazards while also increasing the peptides resistance to oxidative degradation after formulation is complete. It is believed that the presence of the alkyl group at the number 2 position of the Tryptophan increases the stability of the pyrrole ring wherein attack by reactive radicals and active or activated groups occurs.

While it is apparent that the invention herein disclosed is well calculated to fulfill the objects above stated, it will be appreciated that numerous embodiments and modification may be devised by

---

- 12 -

those skilled in the art, and it is intended that the appended claims cover all such modification and embodiments as fall within the true spirit and scope of the present invention.

5

10

15

20

25

30

35

- 13 -

SEQUENCE LISTING

## (1) INFORMATION FOR SEQ ID NO:1

## (i) SEQUENCE CHARACTERISTICS

- 5 (A) LENGTH: 5  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TYPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: Yes

## 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1

Glu His Trp Ser Tyr  
1 5

## (2) INFORMATION FOR SEQ ID NO:2

## 15 (i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 9  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TYPOLOGY: linear

20 (ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: Yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2

Phe Cys Tyr Pro Gln Gln Trp Phe Trp  
1 5

25

## (3) INFORMATION FOR SEQ ID NO:3

## (i) SEQUENCE CHARACTERISTICS

- 30 (A) LENGTH: 4  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TYPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: Yes

## 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3

Leu Arg Pro Gly  
1

---

- 14 -

## (4) INFORMATION FOR SEQ ID NO:4

## (i) SEQUENCE CHARACTERISTICS

- 5 (A) LENGTH: 4  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TYPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: Yes

## 10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4

Lys Val Cys Trp  
1

## (5) INFORMATION FOR SEQ ID NO:5

## (i) SEQUENCE CHARACTERISTICS

- 15 (A) LENGTH: 4  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TYPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

20 (iii) HYPOTHETICAL: Yes

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5

Ala Trp Phe Lys  
1

## 25 (6) INFORMATION FOR SEQ ID NO: 6

## (i) SEQUENCE CHARACTERISTICS

- 30 (A) LENGTH: 6  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TYPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: Yes

## 35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6

Pro Gln Gln Trp Phe Trp  
1 5



- 15 -

## (7) INFORMATION FOR SEQ ID NO: 7

## (i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 10  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TYPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7

Glu His Trp Ser Tyr Trp Leu Arg Pro Gly  
1 5 10

## (8) INFORMATION FOR SEQ ID NO: 8

## (i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 9  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TYPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8

Glu His Trp Ser Tyr Trp Leu Arg Pro  
1 5

## (9) INFORMATION FOR SEQ ID NO: 9

## (i) SEQUENCE CHARACTERISTICS

- (A) LENGTH: 8  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TYPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9

Pro Gln Gln Trp Phe Trp Trp Met  
1 5

- 16 -

## (10) INFORMATION FOR SEQ ID NO:10

## (i) SEQUENCE CHARACTERISTICS

- 5 (A) LENGTH: 6  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TYPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10

10 Arg Trp Phe Trp Leu Met  
1 5

## (11) INFORMATION FOR SEQ ID NO:11

## (i) SEQUENCE CHARACTERISTICS

- 15 (A) LENGTH: 7  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TYPOLOGY: linear

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11

25 Phe Cys Phe Trp Lys Thr Cys  
1 5

## (12) INFORMATION FOR SEQ ID NO: 12

## (i) SEQUENCE CHARACTERISTICS

- 30 (A) LENGTH: 8  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TYPOLOGY: linear, and cyclical  
oxidation product

(ii) MOLECULE TYPE: Peptide

(iii) HYPOTHETICAL: Yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12

35 Phe Cys Tyr Trp Lys Val Cys Trp  
1 5

- 17 -

## (13) INFORMATION FOR SEQ ID NO: 13

- (i) SEQUENCE CHARACTERISTICS  
(A) LENGTH: 6  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TYPOLOGY: linear
- (ii) MOLECULE TYPE: Peptide
- (iii) HYPOTHETICAL: Yes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13

His Trp Ala Trp Phe Lys  
1 5

## (14) INFORMATION FOR SEQ ID NO: 14

- (i) SEQUENCE CHARACTERISTICS  
(A) LENGTH: 10  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TYPOLOGY: linear
- (ii) MOLECULE TYPE: Peptide
- (iii) HYPOTHETICAL: Yes
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14

Glu His Trp Ser Tyr Gly Leu Arg Pro Gly  
1 5 10

CLAIMS

1. Bioactive peptides containing D-Tryptophan  
5 residues wherein at least one D-Tryptophan residue is  
replaced with a D-2-alkylTryptophan having a lower  
alkyl group substituted at the number 2 position, said  
D-2-alkylTryptophan providing increased oxidation  
resistance to the peptide while maintaining  
10 substantially the same pharmacological effect as  
analogous bioactive peptides in which the at least one  
Tryptophan residue is not replaced.

2. The bioactive peptides of claim 1 wherein the  
lower alkyl group includes 1 to 3 carbons.

3. The bioactive peptides of claim 2 wherein the  
lower alkyl group is methyl, ethyl, or isopropyl.

4. Bioactive peptides characterized by the  
formula A-D-2-alk-Trp-B, wherein alk is a lower alkyl  
group;

20 A is SEQUENCE ID NO:1 wherein the Glutamic  
acid residue is pyroGlu; D-Phe-Cys-Phe; D-Phe-Cys-Tyr;  
SEQUENCE ID NO:6 wherein the Proline residue is D-Pro  
and the Tryptophan residues at SEQUENCE positions 4 and  
6 are D-Trp; Arg-D-Trp-N-methyl-Phe; or His; and

25 B is SEQUENCE ID NO:3 wherein the Glycine  
residue is Gly-NH<sub>2</sub>; Leu-Arg-Pro-NHCH<sub>2</sub>CH<sub>3</sub>;  
Lys-Thr-Cys-NHCH(CH<sub>2</sub>OH)CHOH(CH<sub>3</sub>), SEQUENCE ID NO:4  
wherein the Tryptophan residue is Trp-NH<sub>2</sub>; Met-NH<sub>2</sub>;  
Leu-Met-NH<sub>2</sub> or SEQUENCE ID NO:5 wherein the  
30 Phenylalanine residue is D-Phe and the Lysine residue  
is Lys-NH<sub>2</sub> provided that:

when A is SEQUENCE ID NO:1 wherein the  
Glutamic acid residue is pyroGlu, B is SEQUENCE ID NO:3  
wherein the Glycine residue is GlyNH<sub>2</sub> or  
35 Leu-Arg-Pro-NHCH<sub>2</sub>CH<sub>3</sub>;

- 19 -

when A is D-Phe-Cys-Phe, B is Lys-Thr-Cys-NHCH(CH<sub>2</sub>OH)CHOH(CH<sub>3</sub>) wherein the cysteine units are bound to each other by a cyclic disulphide;

when A is D-Phe-Cys-Tyr, B is SEQUENCE ID NO:4 wherein the Tryptophan residue is Trp-NH<sub>2</sub> and the cysteine units are bound to each other by a cyclic disulfide;

when A is SEQUENCE ID NO:6 wherein the Proline residue is D-Pro and the Tryptophan residues at SEQUENCE positions 4 and 6 are D-Trp, B is Met-NH<sub>2</sub>;

when A is Arg-D-Trp-N-methyl-Phe, B is Leu-Met-NH<sub>2</sub>; and

when A is His, B is SEQUENCE ID NO:5 wherein the Phenylalanine residue is D-Phe and the Lysine residue is Lys-NH<sub>2</sub>; wherein said peptides provide increased resistance to ionized sterilization, reactive radicals, and oxidation while maintaining a similar pharmacological effect as compared to analogous D-Tryptophan containing peptides not containing at least one D-2-alkylTryptophan.

5. The bioactive peptides of claim 4 wherein the lower alkyl group includes 1 to 3 carbon atoms.

6. The bioactive peptides of claim 5 wherein the alkyl group is methyl, ethyl, or isopropyl.

7. The bioactive peptides according to claim 4 wherein said peptide is SEQUENCE ID NO: 7 wherein the Glutamic acid residue at SEQUENCE position 1 is Pyro-Glu, the Tryptophan residue at SEQUENCE position 6 is D-2-methyl-Trp, and the Glycine residue at SEQUENCE position 10 is Gly-NH<sub>2</sub>; SEQUENCE ID NO:8 wherein the Glutamic Acid residue at SEQUENCE position 1 is Pyro-Glu, the Tryptophan residue at SEQUENCE position 6 is D-2-methyl-Trp, and the Proline residue at SEQUENCE position 9 is Pro-NHCH<sub>2</sub>CH<sub>3</sub>; SEQUENCE ID NO:9 wherein the Proline residue at SEQUENCE position 1 is D-Proline, the Tryptophan residues at SEQUENCE positions 4 and 6

- 20 -

are D-Trp, the Tryptophan residue at SEQUENCE position 7 is D-2-ethyl-Trp, and the Methionine residue at SEQUENCE position 8 is Met-NH<sub>2</sub>; SEQUENCE ID NO:10 wherein the Tryptophan residue at SEQUENCE position 2 is D-Trp, the Phenylalanine residue at SEQUENCE position 3 is N-methyl-Phe, the Tryptophan residue at SEQUENCE position 4 is D-2-isopropyl-Trp, and the Methionine residue at SEQUENCE position 6 is Met-NH<sub>2</sub>; SEQUENCE ID NO:13 wherein the Tryptophan residue at SEQUENCE position 2 is D-2-methyl-Trp, the Phenylalanine residue at SEQUENCE position 5 is D-Phe, and the Lysine residue at SEQUENCE position 6 is Lys-NH<sub>2</sub>.

8. The bioactive peptides according to claim 4 wherein said peptide is SEQUENCE ID NO:11 wherein the Phenylalanine residue at SEQUENCE position 1 is D-Phe, the Tryptophan residue at SEQUENCE position 4 is D-2-methyl-Trp, and the Cysteine residue at SEQUENCE position 7 is Cys-NHCH(CH<sub>2</sub>OH)CHOHCH<sub>3</sub>, and wherein the cysteine units are bound to each other by means of a cyclic disulphide bond; or SEQUENCE ID NO: 12 wherein the Phenylalanine residue at SEQUENCE position 1 is D-Phe, the Tryptophan residue at SEQUENCE position 4 is D-2-methyl-Trp, and the Tryptophan residue at SEQUENCE position 8 is Trp-NH<sub>2</sub>, wherein the cysteine units are bound to each other by means of a cyclic disulphide bond.

9. Bioactive peptides containing D-2-alkylTryptophan residues wherein said peptides are SEQUENCE ID NO:7 wherein the Glutamic acid residue at SEQUENCE position 1 is Pyro-Glu, the Tryptophan residue at SEQUENCE position 6 is D-2-methyl-Trp, and the Glycine residue at SEQUENCE position 10 is Gly-NH<sub>2</sub>; SEQUENCE ID NO:8 wherein the Glutamic Acid residue at SEQUENCE position 1 is Pyro-Glu, the Tryptophan residue at SEQUENCE position 6 is D-2-methyl-Trp, and the

- 21 -

Proline residue at SEQUENCE position 9 is Pro-NHCH<sub>2</sub>CH<sub>3</sub>;  
SEQUENCE ID NO:9 wherein the Proline residue at  
SEQUENCE position 1 is D-Proline, the Tryptophan  
residues at SEQUENCE positions 4 and 6 are D-Trp, the  
5 Tryptophan residue at SEQUENCE position 7 is D-2-ethyl-  
Trp, and the Methionine residue at SEQUENCE position 8  
is Met-NH<sub>2</sub>; SEQUENCE ID NO:10 wherein the Tryptophan  
residue at SEQUENCE position 2 is D-Trp, the  
Phenylalanine residue at SEQUENCE position 3 is N-  
10 methyl-Phe, the Tryptophan residue at SEQUENCE position  
4 is D-2-isopropyl-Trp, and the Methionine residue at  
SEQUENCE position 6 is Met-NH<sub>2</sub>; SEQUENCE ID NO:11  
wherein the Phenylalanine residue at SEQUENCE position  
1 is D-Phe, the Tryptophan residue at SEQUENCE position  
15 4 is D-2-methyl-Trp, and the Cysteine residue at  
SEQUENCE position 7 is Cys-NHCH(CH<sub>2</sub>OH)CHOHCH<sub>3</sub>, and  
wherein the cysteine units are bound to each other by  
means of a cyclic disulphide bond; SEQUENCE ID NO: 12  
wherein the Phenylalanine residue at SEQUENCE position  
20 1 is D-Phe, the Tryptophan residue at SEQUENCE position  
4 is D-2-methyl-Trp, and the Tryptophan residue at  
SEQUENCE position 8 is Trp-NH<sub>2</sub>, wherein the cysteine  
units are bound to each other by means of a cyclic  
disulphide bond; and SEQUENCE ID NO:13 wherein the  
25 Tryptophan residue at SEQUENCE position 2 is D-2-  
methyl-Trp, the Phenylalanine residue at SEQUENCE  
position 5 is D-Phe, and the Lysine residue at SEQUENCE  
position 6 is Lys-NH<sub>2</sub>.

10. A method for prolonging the pharmacological  
30 activity of a bioactive peptide containing one or more  
D-Tryptophan residues which comprises replacing at  
least one D-Tryptophan residue of said peptide with a  
D-2-alkylTryptophan residue so as to increase said  
peptides resistance to degradation by oxygen, reactive  
35 radicals and other reactive groups while maintaining a  
similar biological effect as compared to analogous

---

- 22 -

bioactive peptides wherein the Tryptophan residue is not so replaced.

11. A method for preserving the pharmacological effectiveness of a bioactive peptide containing one or  
5 more Tryptophan residues which is to be sterilized or exposed to active or activated groups during manufacture which comprises replacing at least one Tryptophan residue of said peptide with a D-2-alkylTryptophan residue so as to increase the  
10 resistance of said peptide to the effects of sterilization or exposure to active or activated groups as compared to analogous bioactive peptides wherein the at least one Tryptophan residue is not so replaced.

12. The method of claim 11 wherein the bioactive  
15 peptide is sterilized by exposure to ionizing radiation.

13. The method of claim 11 wherein the peptide is exposed to the active or activated groups present in controlled delivery substances based on polylactic-  
20 polyglycolic acid polymers.

25

30

35



# INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 91/00727

|  |  |  |                       |                        |                  |                |
|--|--|--|-----------------------|------------------------|------------------|----------------|
| <b>I. CLASSIFICATION OF SUBJECT MATTER</b> (if several classification symbols apply, indicate all) *<br>According to International Patent Classification (IPC) or to both National Classification and IPC<br>IPC <sup>5</sup> : C 07 K 7/20, 7/26, 7/06, A 61 K 37/24, 37/43   |  |  |                       |                        |                  |                |
| <b>II. FIELDS SEARCHED</b><br><div style="text-align: right; font-size: small;">Minimum Documentation Searched *</div> <table style="width: 100%; border: none;"> <tr> <td style="width: 30%; border: none;">Classification System  </td> <td style="border: none;">Classification Symbols</td> </tr> <tr> <td style="border: none; padding: 5px;">IPC<sup>5</sup></td> <td style="border: none; padding: 5px;">C 07 K, A 61 K</td> </tr> </table> <div style="text-align: center; font-size: x-small; margin-top: 5px;">Documentation Searched other than Minimum Documentation<br/>to the extent that such Documents are Included in the Fields Searched *</div>   |  |  | Classification System | Classification Symbols | IPC <sup>5</sup> | C 07 K, A 61 K |
| Classification System  | Classification Symbols   |  |                       |                        |                  |                |
| IPC <sup>5</sup>   | C 07 K, A 61 K   |  |                       |                        |                  |                |
| <b>III. DOCUMENTS CONSIDERED TO BE RELEVANT</b> †  |  |  |                       |                        |                  |                |
| Category *   | Citation of Document, †† with indication, where appropriate, of the relevant passages ‡  | Relevant to Claim No. ‡‡   |                       |                        |                  |                |
| A  | The New England Journal of Medicine, 29<br>March 1990, R.M. Silver et al.: "Sclero-<br>derma, fasciitis, and eosinophilia<br>associated with the ingestion of trypto-<br>phan", pages 874-881<br>see the whole document<br>cited in the application<br>--- | 1-3  |                       |                        |                  |                |
| A  | US, A, 3316260 (TSUNG-YING SHEN)<br>25 April 1967<br>see column 2, lines 65-72; column 3,<br>lines 26-46; examples 1-55; claims 1-4<br>---   | 1-3  |                       |                        |                  |                |
| A  | Chem. Pharm. Bull., volume 27, no. 8, 1979,<br>Y. Yabe et al.: "Synthesis and biologi-<br>cal activity of LH-RH Analogs subs-<br>tituted by alkyltryptophans at position<br>3", pages 1907-1911<br>see the whole document<br>---                           | 1-3, 10, 11  |                       |                        |                  |                |
| A  | Endocrine Reviews, volume 7, no. 1, 1986,<br>The Endocrine Society (US), M.J. Karten<br>et al.: "Gonadotropin-releasing hormone<br>/   | 4, 7, 9  |                       |                        |                  |                |
| <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;">           * Special categories of cited documents: †<br/>           "A" document defining the general state of the art which is not<br/>           considered to be of particular relevance<br/>           "E" earlier document but published on or after the International<br/>           filing date<br/>           "L" document which may throw doubt on priority claim(s) or<br/>           which is cited to establish the publication date of another<br/>           citation or other special reason (as specified)<br/>           "O" document referring to an oral disclosure, use, exhibition or<br/>           other means<br/>           "P" document published prior to the International filing date but<br/>           later than the priority date claimed         </div> <div style="width: 45%;">           "T" later document published after the International filing date<br/>           or priority date and not in conflict with the application but<br/>           cited to understand the principle or theory underlying the<br/>           invention<br/>           "X" document of particular relevance; the claimed invention<br/>           cannot be considered novel or cannot be considered to<br/>           involve an inventive step<br/>           "Y" document of particular relevance; the claimed invention<br/>           cannot be considered to involve an inventive step when the<br/>           document is combined with one or more other such docu-<br/>           ments, such combination being obvious to a person skilled<br/>           in the art.<br/>           "Δ" document member of the same patent family         </div> </div> |  |  |                       |                        |                  |                |
| <b>IV. CERTIFICATION</b>   |  |  |                       |                        |                  |                |
| Date of the Actual Completion of the International Search<br><br>4th June 1991   |  | Date of Mailing of this International Search Report<br><br>17 SEP 1991 |                       |                        |                  |                |
| International Searching Authority<br><br>EUROPEAN PATENT OFFICE  |  | Signature of Authorized Officer<br>MISS I. TAZELAAR                    |                       |                        |                  |                |

| III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET) |   |                       |
|--|---|-----------------------|
| Category *   | Citation of Document, " with indication, where appropriate, of the relevant passages  | Relevant to Claim No. |
|  | <p>analog design. Structure-function studies toward the development of agonists and antagonists: Rationale and perspective", pages 44-66<br/>see page 46</p> <p>---</p>   |                       |
| A  | <p>EP, A, 0203031 (THE ADMINISTRATORS OF THE TULANE UNIVERSITY EDUCATIONAL FUND)<br/>26 November 1986<br/>see the whole document</p> <p>---</p>   | 4,8,9                 |
| A  | <p>Proc. Natl. Acad. Sci. USA, volume 83, 1986, Y. Torrens et al.: "Substance P receptors in primary cultures of cortical astrocytes from the mouse", pages 9216-9220<br/>see the whole document</p> <p>---</p> | 4,7,9                 |
| A  | <p>EP, A, 0083864 (BECKMAN INSTRUMENTS)<br/>20 July 1983<br/>see the whole document</p> <p>-----</p>  | 4,7,9                 |

**ANNEX TO THE INTERNATIONAL SEARCH REPORT  
ON INTERNATIONAL PATENT APPLICATION NO.**

EP 9100727

SA 46621

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 19/08/91. The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
|---|---------------------|----------------------------|---------------------|
| US-A- 3316260                             |                     | BE-A- 688765               | 31-03-67            |
|   |                     | CH-A- 468378               |                     |
|   |                     | CH-A- 476724               | 15-08-69            |
|   |                     | CH-A- 487140               | 15-03-70            |
|   |                     | DE-A- 1543840              | 21-05-70            |
|   |                     | DE-A, B, C 1620222         | 06-04-72            |
|   |                     | FR-M- 5110                 | 29-05-67            |
|   |                     | FR-M- 6717                 | 17-02-69            |
|   |                     | FR-M- 6041                 | 20-05-68            |
|   |                     | FR-A- 1458096              |                     |
|   |                     | FR-A- 1497423              |                     |
|   |                     | FR-A- 1602629              | 04-01-71            |
|   |                     | GB-A- 1080470              |                     |
|   |                     | GB-A- 1140803              |                     |
|   |                     | GB-A- 1169014              | 29-10-69            |
|   |                     | NL-A- 6415318              | 02-07-65            |
|   |                     | NL-A- 6614056              | 25-04-67            |
|   |                     | NL-A- 6615029              | 25-04-67            |
| EP-A- 0203031                             | 26-11-86            | US-A- 3419555              |                     |
|   |                     | US-A- 3470189              | 30-09-69            |
|   |                     | US-A- 4650787              | 17-03-87            |
|   |                     | US-A- 4725577              | 16-02-88            |
|   |                     | AU-B- 600895               | 30-08-90            |
| EP-A- 0083864                             | 20-07-83            | AU-A- 5633886              | 30-10-86            |
|   |                     | JP-A- 61293997             | 24-12-86            |
|   |                     | US-A- 4410512              | 18-10-83            |
|   |                     | US-A- 4410513              | 18-10-83            |
|   |                     | US-A- 4411890              | 25-10-83            |
|   |                     | AU-B- 549053               | 09-01-86            |
|   |                     | CA-A- 1242435              | 27-09-88            |
|   |                     | WO-A- 8302272              | 07-07-83            |

EPO FORM 7001

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82